PARN AS A BIOMARKER AND THERAPEUTIC TARGET

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/715,688 filed Aug. 7, 2018, which is incorporated herein by reference in its entirety.

GOVERNMENT INTEREST

[0002] This invention was made with government support under grant number GM045443, awarded by National Institutes of Health. The United States government has certain rights in the invention.

TECHNICAL FIELD

[0003] The invention relates to medical uses and methods for modulating gene expression and treating cancer using poly(A)-specific ribonuclease (PARN) inhibitors, and to methods and uses for overcoming resistance of cancer cells to chemotherapy, including selecting PARN inhibitors for use in treating cancer in a subject, both in the initial selection of PARN inhibitors and for addressing the development of acquired drug resistance that occur in the course of treatment.

BACKGROUND OF INVENTION

[0004] The adenylation of 3' ends of cellular RNAs by poly(A) polymerases plays a critical role in the function and stability of both mRNAs and non-coding RNAs. PARN is a processive mammalian poly(A)-specific ribonuclease that has previously been shown to remove poly(A) tails from the 3' ends of mRNAs. Recent work has shown that PARN also regulates the stability of several ncRNAs in mammalian cells, including scaRNAs, human telomerase RNA (hTR), piRNAs and Y RNAs, suggesting that the deadenylation activity of PARN is important for regulating the stability of a variety of RNAs in mammalian cells.

[0005] miRNAs are small 21-23 nt non-coding RNAs that regulate gene expression in eukaryotic cells through base pairing with their target mRNAs. miRNAs are transcribed as long primary transcripts (pri-miRNA), which are trimmed by the endonuclease Drosha to generate the precursor miRNA (pre-miRNA) containing the miRNA stem-loop. The pre-miRNA is subsequently cleaved by Dicer to generate the mature miRNA, which assembles with Argonaute and GW182 along with other proteins to form the RNA-induced silencing complex (RISC). While the role of miRNAs in regulating gene expression is well studied, the mechanism(s) that globally regulates the stability of miRNAs in mammalian cells are not fully understood. Previous work has suggested that XRN2-mediated 5' to 3' degradation can regulate the stability of some miRNAs in model organisms. More recent work has shown that Tudor S/N mediated endonucleolytic cleavage can also regulate the stability of some miRNAs in mammalian cells.

[0006] miRNAs are known to be modified by non-templated U or A additions at the 3' end in diverse cell types and organisms. In plants, Hen1-mediated 3' end methylation of the 2'-OH moiety has been shown to protect endogenous plant siRNAs and miRNAs from uridylation and degradation by SND1. In black cottonwood plant, adenylation of the 3' end is a feature of miRNA degradation products, and adenylation can also reduce the degradation of plant miRNAs. In the alga *Chlamydomonas*, Mut68 uridylates the 3' ends of endogenous siRNAs and miRNAs, suggesting a conserved function of 3' end modification of small RNAs in different organisms.

[0007] The best studied example of 3' end non-templated addition in mammalian miRNAs is the uridylation of the let-7 pre-miRNA by non-canonical uridylases TUT4/TUT7. Tut4/Tut7 are recruited by the RNA binding protein LIN28 to the pre-let-7, which leads to polyuridylation of the pre-let-7 3' end and affects its processing into mature let-7, thereby playing a role in regulating let-7 miRNA levels and function in animal development. It has also been proposed that monouridylation of some let-7 pre-miRNAs as opposed to polyuridylation is important for their processing to mature let-7 miRNA in HeLa cells, suggesting that the activity of Tut4/Tut7 may be regulated in mammalian cells to maintain a balance between let-7 processing and degradation. Uridylation of pre-let-7 leads to the recruitment of the 3' to 5' exonuclease DIS3L2, and DIS3L2 degrades polyuridylated pre-let-7 in undifferentiated stem cells. Uridylation of pre-miRNAs and miRNAs has also been shown to occur on other families of miRNAs in diverse cell types, suggesting that uridylation of pre-miRNAs and mature miRNAs is a general feature of miRNA regulation.

[0008] Adenylation at the 3' end has also been shown to occur for some miRNAs, although it is suggested to be less frequent compared to uridylation. The best understood example of miRNA adenylation is GLD2-mediated monoadenylation of miR-122, which enhances the stability and function of miR-122 in mammalian cells. Similarly, monoadenylation of the 3' end by GLD2 also enhanced the stability of some other miRNAs in human fibroblasts. In contrast, PAPD5-mediated adenylation has been proposed to destabilize miR-21 in human cancer cell lines. In *Drosophila*, wisp-mediated adenylation of the 3' end destabilizes maternal miRNAs in eggs and is an important step in maternal miRNA clearance. For monoadenylated miR-122 in liver cells, PARN has been proposed to be the enzyme responsible for the degradation of miR-122 when it is adenylated by GLD2. However, the mechanisms that regulate miRNA 3' end adenylation and deadenylation, and the role of PARN in this process, are not well understood.

[0009] Dyskeratosis Congenita (DC) is caused by genetic defects in components of the telomerase holoenzyme in human cells and leads to bone marrow failure and cancer. While most mutations associated with DC pathogenesis are in genes important for human telomerase RNA (hTR) assembly (DKC1) or telomerase RNA stability (TERC), mutations in PARN were shown to cause a severe form of DC known as Hoyeraal-Hreidarsson syndrome, which causes abnormally short telomeres and congenital. Subsequently, it was shown that loss of PARN leads to defective 3' end maturation of hTR, leading to oligoadenylation by PAPD5 and 3' to 5' degradation by EXOSC10 in the nucleus, or cytoplasmic export and decapping and 5' to 3' degradation by DCP2/XRN1. While loss of telomerase RNA function explains telomere shortening in DC patients, it doesn't explain the pleiotropic and severe phenotype of the disease caused by PARN mutations.